THESIS ABSTRACT

Studies on Plant Bio-Molecules and Their Application Potential

Winged bean (*Psophocarpus tetragonolobus*) seeds are rich source of Kunitz-type serine protease inhibitors of about 20 kDa in size with two disulfide bridges. In this work structural changes of winged bean trypsin inhibitor (WbTI-2) and bi-functional chymotrypsin/ trypsin inhibitor (WBCTI) have been studied as a function of temperature. These two proteins completely retained inhibitory properties against trypsin and/ or chymotrypsin even after heating at 70°C. On the basis of circular dichroism studies it appeared that WBCTI and WbTI-2 maintained their canonical structure up to 70°C. But the activity and stability of the secondary structures were found to decrease drastically in presence of dithiothreitol, indicating the role of two disulfide bonds for additional stability of these proteins.

When insecticidal and growth inhibitory potential were evaluated, both the proteins have shown reasonable inhibition of mid-gut proteases of *Helicoverpa armigera*. In artificial feeding trial, addition of WBCTI and WbTI-2 in diet resulted in significant growth retardation of *H. armigera* larva. WBCTI also caused delayed pupae formation and higher mortality in *H. armigera* larvae. Based on this observation, the possibility of WBCTI as an effective insecticidal lead molecule has been studied by generating transgenic tobacco plants. Transgenically expressed WBCTI has shown reasonable inhibition of mid-gut proteases of *H. armigera*. During infestation of wild type and transgenic tobacco plants with *H. armigera* larva, transgenic plants were resistant to insect attack to a great extent compared to the wild type plants. This finding supported the deployment of WBCTI as a suitable candidate gene from a non-host plant for producing stress-tolerant transgenic plant.

In the final part of the work, the concept of protein engineering was explored by appending the amylase inhibitory property in WBCTI to convert it to an amylase-trypsin-chymotrypsin trifunctional inhibitor (TFI). An amylase-inhibitory nona-peptide, representing the N-terminus of ragi bi-functional inhibitor (RBI), was attached with WBCTI through PCR. This newly engineered protein was completely functional as it inhibited alpha amylase, trypsin and chymotrypsin. Interestingly it has also shown reasonable inhibition of the mid-gut proteases of *H. armigera*. So it can be concluded that TFI may become an efficient insecticidal lead molecule, as a single defensive gene product, to combat with the protein as well as carbohydrate digestive systems of the pests.

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